REMARKS

Rejection of claims under 35 U.S.C. 112

Claims 1-3, 5-7, 11, 12, 16-20, 24, 25, 27, 27-31, 34-36 and 38-42 have been rejected under U.S.C. 112 first and second paragraph. Applicants have amended the claims to obviate the rejections. The amended claims 1 and 39 more clearly indicate the muscle cells to which the nucleic acid is delivered in relation to the vessel into which the nucleic acid is inserted. The amended claims also more clearly indicate which vessels are occluded by the applied pressure.

Methods for occluding blood flow by applying external pressure are well know in the art. Tourniquets are a well known method of stopping blood loss in emergency medical situations. Sphygmomonameters, used to measure blood pressure, measure the pressure (applied externally against the skin) needed to stop blood flow to a limb. Pressure points, places where one applies external pressure to compress internal vessels to occlude blood flow and thus prevent blood loss, are taught in first aid classes. No experimentation is required for one skilled in the art to know where to apply pressure to occlude blood flow through a given vessel. The specification clearly indicates that pressure is applied, not simply for the sake of pushing against skin, but for affecting blood flow though vessels. Thus, Applicants contemplate applying pressure to the skin and not only to applying a cuff around the limb.

The desired outcome of gene therapy may be the long term expression of a protein that was not previously expressed as an endogenous protein in the patient. Because the transgenic protein is not an endogenous protein, there arises the potential of an immune response against the transgenic protein. An analogous situation arises in organ transplantation. Rejection of the organ is an immune response which is suppressed by the administration of immunosuppressive treatment. Patients who receive organ transplants remain on immunosuppressive drugs for the rest of their lives – long term immunosupressive treatment. Acute conditions, such as severe allergic reactions, anaphylactic shock, and certain toxins are also treated with immunosupprossive drugs. Rejection of a transgenic gene product and the cells expressing the transgenic gene would be treated in a same manner as rejection of a transplanted organ. Immunosuppressive drugs may have to be given to the patient for as long as the transgene is expressed. It is also well known in the field of gene therapy that immune response to a component of the delivery system may also be a potential limiting factor in effective gene delivery. Short term, or transient, immunosuppression may be required for efficient gene therapy in such cases. Clearly there is extensive knowledge in the use of immunosuppression for both chronic and acute medical conditions and so the level of skill in the art is extraordinarily high. We demonstrate the potential benefits of immunosuppression in enhancing gene therapy in example 5 of the specification (page 28-29).

The Office Action states on page 3 that "limitation of 'function is not affected by the delivery process'" does not have support in the specification. Applicants respectfully disagree. Evidence for maintaining function of the limb following the procedure can be found in the following sections of the specification: page 22, lines 15-27; page 25 lines 17-25, page 28, lines 6-23. Applicants have clearly stated that: no ischemic damage is observed in the tissue following the procedure, there is no evidence of damage to nerves in the limbs, the animals tolerated the procedure well, no discomfort was caused to the animals beyond normal surgical recovery, serum enzyme levels (a measure of toxicity) were minimally elevated and returned to normal quickly, and there is no histological sign of pathology.

The method taught by the Applicants is differentiated from the method taught by Milas et al 1997 in three distinct ways. First, while Fig. 1A of Milas et al appears to show a tourniquet placed around a rat leg, the text on page 2198, column 2 indicates that the tourniquet is in fact placed "underneath the inguinal ligament." Second, the method of Milas et al teach perfusion of the limb requiring surgical cannulation of two vessels. Milas et al further teach perfusion with normal saline both before and after perfusion of the adenovirus. Milas et al state on page 2202, paragraph 1 that "cannulation of the femoral vein with resultant brisk outflow is critical for the success of the procedure... ." Finally, Milas et al teach the ligation of the vessels distal to the cannulation sites, page 2199, paragraph 1. In contrast Applicants teach injection into a single blood vessel and a non-invasive cuff without ligation of the vessel after the procedure. Applicants believe that the amended claims do not encompass the methods taught by Milas and Ye.

The Office Action states on page 5 that it can not be determined how delivery to specific muscle cells (claims 11, 12, 17, 24, 25, and 29-31) is affected by the location of the blood vessel injected. Applicants respectfully disagree.

The specification indicates that the polynucleotide is injected into an afferent or efferent vessel of the target tissue or organ. In other words, the vessel is selected by identifying the vessel or vessels that deliver blood to or carry blood away from the target tissue. Applicants furthermore believe that claims 11, 12, 17, 24, 25, and 29-31 are not restricting delivery to the explicitly named muscle cells. The named muscles are present in the arm or leg and therefore practice of the invention results in delivery to the claimed muscle cells.

The Office Action states on page 6 that the specification does not correlate the results obtained with naked plasmid DNA to any other vector. Applicants submit data demonstrating such a correlation (see accompanying 2 pages). These examples, using a similar process, demonstrate delivery of DNA/polycation complexes, small double stranded RNA oligonucleotides, and viral particles.

The Office Action states on page 6 that applicants' specification teaches only delivery of a polynucleotide linked to a promoter and not to any polynucleotide as broadly claimed. This does not appear to be a reasonable argument since the physical nature of a polynucleotide is largely independent of the sequence of the polynucleotide.

Blocking polynucleotides are simply polynucleotides that bind to other cellular factors including other polynucleotides, such as RNA, rather than being utilized as templates for transcription by polymerases. Blocking polynucleotides, or antisense nucleotides, are not significantly different from expression cassettes either physically or chemically. There is considerable knowledge in the art for inhibiting expression of a gene with another nucleotide sequence. A search of PubMed for articles in which antisense or RNA interference is present in the title yields 4975 and 171 hits respectively. As such, no undue experimentation is needed to determine the metes and bounds of a blocking nucleic acid.

The Office Action states on page 8 that "the mere delivery of polynucleotide to any cell does not have a disclosed use after delivering the DNA to a skeletal muscle cell. The method should result in expression of a protein in skeletal muscle cell." Applicants respectfully disagree.

Applicants point to the extensive evidence in the scientific literature of nucleic acid delivery to a cell for the purpose of inhibiting expression of an endogenous gene (see reviews: Wang et al. Antisense anticancer oligonucleotide therapeutics. Curr Cancer Drug Targets 2001 1(3):177-196; Mercatante et al. Modification of alternative splicing by the antisense oligonucleotides as a potential chemotherapy for cancer and other diseases. Curr Cancer Drug Targets 2001 1(3):211-230; Cho-Chung. Antisense DNAs as targeted therapeutics for cancer: no longer a dream. Curr Opin Investig Drugs 2002 3(6):934-939; Shuey et al. RNAi: gene-silencing in therapeutic intervention. Drug Discov Today 2002 7(20):1040-1046; and Shi. Mammalian RNAi for the masses. Trends Genet 2003 19(1):9-12). The delivered nucleic acid itself can inhibit expression of the endogenous gene as in antisense and RNA interference mechanisms of inhibition. Alternatively, the nucleic acid can be expressed to produce an RNA transcript that inhibits expression of an endogenous gene. In these examples, the delivered nucleic acid need not result in expression of a protein to alter the endogenous properties of the cell.

Claim 5 has been largely incorporated into claim 1 and has been canceled. Claims 6, 18, 19, 20, 24, 27, 28, and 30 have been amended to correct the indefinite rejections. Claims 11, 12, 16, 17, 34 and 35 have been amended to make them dependent on the appropriate claims. Claims 11, 12, 16, 17, 30, and 31 have been amended to correct the Markush group rejections. Claim 27 has been canceled, and claim 30 has been amended to depend from claim 6. Claims 11 and 12 have been amended to remove the abbreviations, spf. and prof.

The Office Action states on page 9 that the metes and bounds of "cuff" can not be determined. Applicants believe they have clearly defined a cuff, for the purposes of the claims, as: a device applied exterior to the mammal's skin that touches the skin in a non-invasive manner. As stated above, Applicants believe that the art of compressing blood vessels using pressure exerted against the surface of a mammal from an external position is well established. Therefore, Applicants believe that the metes and bounds of the term "cuff" are readily envisioned.

The Office Action states that the metes and bounds of claim 37 can not be determined. Applicants point out that claim 37 has previously been canceled.

The Office Action states on page 10 that the metes and bounds of non-vascular parenchymal cells can not be determined. All of the cells of blood vessels are vascular cells. Since the applicants clearly state non-vascular parenchymal cells, the distinguishing cells of a blood vessel are irrelevant.

Rejection of claims under 35 U.S.C. 102

Claims 1, 3, 5, 6, 11, 12, 16, 17, 24, 25, 27-31, 34-39 and 38-42 have been rejected under 35 U.S.C. 102 as being anticipated by Milas et al 1997. Applicants have amended the claims to obviate the rejections. Applicants believe that the amended claims do not encompass the method taught by Milas et al. for the reasons stated in response to the §112 rejections.

The Office Action states on page 12 that occluding blood vessels is immunosuppression because blood cells are prevented from flowing through the area. However, since the tissues are not perfused to remove blood cells, immune cells remain present in the area and the act of

occluding blood flow does not constitute immunosupression. As discussed above, the use of immunosuppression in medical procedures is common.

Claims 1-42 are rejected as being anticipated by Von der Leyen et al 1999. Applicants have amended to claims to more distinctly differentiate their method from the method of Von der Leyen. Applicants note, however, that a sphygmomanometer was used by Von der Leyer, not to increase pressure in the artery, but to monitor pressure in an isolated arterial segment. The sphygmomanometer was not placed around a limb. Furthermore, while Von der Leyen did observe delivery of nucleic acid to multiple layers of the artery (page 2362, column 1, lines 16-21) which is surrounded by skeletal muscle in vivo, they did not observe delivery out of the vessel to skeletal muscle cells.

Rejection of claims under 35 U.S.C. 103

Claims 1, 3, 5, 6, 11, 12, 16, 17, 27, 28, 30, 31, 34-36 and 38-42 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Wolff '387 in view of Milas et al 1977. Claims 1, 3, 5, 6, 11, 12, 16, 17, 24, 25, 27-31, 34-36 and 38-42 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Budker et al. 1998 in view of Milas et al 1977. Applicants believe they have developed a significant advance over the technique taught by Budker and Wolff. The Applicants' method is less invasive, yet results in efficient delivery of polynucleotides to skeletal muscle cells.

Applicants argue that the method taught by Milas actually failed to result in delivery of adenovirus to skeletal muscle cells despite prolonged perfusion of the virus. Furthermore, the method of Milas failed to retain the virus in the limb vasculature as evidence by the 7.5% leakage rate and infection if liver cells. Finally, the method of Milas required major surgery and an invasive placement of the tourniquet (under the inguinal ligament).

Claims 1, 3, 5, 6, 11, 12, 16, 17, 24, 24, 27-31, 34-36 and 38-42 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Milas et al 1977 in view of Nabel '488. Applicants have amended the claims to obviate the rejections. Applicants believe that the amended claims do not encompass the method taught by Milas et al. for the reasons stated in response to the §112 rejections.

Double patenting

For the reasons stated in response to the §103 rejections, Applicants' believe that the instant application provides a significant advance over Wolff '387 and is not obvious in view of Milas et al.

The Examiner's objections and rejections are now believed to be overcome by this response to the Office Action. In view of Applicants' amendment and arguments, it is submitted that claims 1-3, 6, 7, 11-12, 16-20, 24, 25, 28-31, 34-36 and 38-42 should be allowable and Applicants respectfully request an early notice to such effect.

Respectfully submitted,

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I hereby certify that this correspondence is being sent by facsimile transmission to: (703) 308-4242, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450; in this date:

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Signature